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(57) Abstract

The invention provides a process for producing highly purified citric acid. A citric acid broth is produced by fermentation (0, 1, 2) from a carbohydrate substrate (sucrose, saccharose, glucose, dextrose, hydrolysed starch) using a selected strain of Aspergillus niger. Fermentation is commenced at low pH, e.g. 2.8, to suppress formation of noxious acids (in particular oxalic acid). Fermentation is continued to very low residual sugar contents, below 2000 mg/l, e.g. 600 mg/l and high ratios of citric acid to residual sugar, e.g. 275 : 1. After coarse filtration (10, 11, 15) the free citric acid is ultrafiltrated (16), using a membrane stable at low pH (1.2) and having a separation cut at a molecular weight of about 10 000 dalton. After cation (22) and anion exchange (26) and activated carbon treatment (21) citric acid is recovered and purified by repeated crystallisation (40, 42, 50, 53). For maximum product purity a sidestream (44, 45, 80) may be withdrawn from the mother liquor of the first crystallisation stage and subjected to one or more treatments, e.g. purification and recycling. The new A. niger strain CCM 8210 according to the invention has the ability to start the fermentation at a pH at least as low as 4, e.g. 2.8 and continue the fermentation until the pH of the substrate has dropped at least as low as pH 1.4 at which stage the sugar level has dropped to not more than 1000 mg/l and the ratio of citric acid to residual sugar may be as high as 275 : 1 - 300 : 1. The broth so produced is processed as aforesaid.

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Process, apparatus and microorganism strain for the manufacture of citric acid

TECHNICAL FIELD OF THE INVENTION

The present invention relates to a process for producing purified citric acid or citric acid concentrate which comprises producing by fermentation a raw aqueous citric acid solution and subjecting it to a preliminary physical separation of citric acid from the bulk of its major, mainly macromolecular contaminants and optional subsequent purification steps. The present invention also relates to a new strain of Aspergillus niger, capable of fermenting carbohydrate substrates to citric acid and forming a fermentation broth suitable for the above process. The invention further relates to an apparatus for carrying out the process, comprising a fermentation stage and optional separation means capable of producing a raw citric acid solution and a downstream preliminary separation stage wherein citric acid is separated from the bulk of its major mainly macromolecular contaminants.

Such process and apparatus has been known and has been in commercial use.

BACKGROUND PRIOR ART

According to the prior art (US Patent 1964, 3.119.821; Rohr, M., Kubicek, P.C., Kominek, J.: Citric Acid. Biotechnology (Reed, G. and Rehm, H.J. eds.) 1983 Vol III pp 419-454, Verlag Chemie Weinheim, Weinheim; Milsom, P.E.: Organic Acids by fermentation, especially citric acid. Food Biotechnology 1. (King, R.D., Cheetham, P.S.J. eds.) 1987 pp 273-307, Elsevier Applied Science, Amsterdam) carbohydrate substrates are fermented to raw aqueous citric acid solutions using a variety of micro-organisms, including yeasts, but in particular Aspergillus niger conidia. This is followed by sieve and press filtration mainly to remove the mycelium. This is followed by the step of preliminary separation of citric acid from the bulk of its major, mainly macromolecular contaminants in particular proteins. Other bothersome impurities which have to be removed in the more classical process in order to arrive at a citric acid product of high grade (e.g. pharmaceutical grade such as BP 93) are residual sugar contents and

acids. Oxalic acid contamination is particularly bothersome because of its toxicity. In the classical prior art process the further purification is primarily performed by the addition of lime which results in the precipitation of calcium citrate. It may also involve steps for the selective precipitation of proteinaceous substances. The precipitated calcium citrate must then be acidified e.g. with sulphuric acid to redissolve citric acid which is then subjected to successive crystallisation steps to recover a purified citric acid. This prior art process suffered from the disadvantage that it was cumbersome, required the employment of relatively large amounts of lime and sulphuric acid and resulted in a problem of disposing of the relatively large amounts of gypsum, dumping of which presents an environmental problem.

The inclusion in classical prior art processes of activated carbon adsorption (mainly to improve colour) and the removal of some ionic impurities by ion exchanger was known as such.

In the past molasses from cane or beet sugar production have usually served as the basis for the fermentation liquors.

Various proposals have been made to remove high molecular weight impurities of citric acid fermentation broths by physical means and in particular by ultrafiltration or reverse osmosis using membranes. Some suitable membranes (based on PVC), stable at pH 1 to 13 at temperatures up to 65% are disclosed in Czech Author's Certificate No 254 223. The separation cut in respect of molecular weight is not disclosed.

In accordance with US patent 4,855,494 and corresponding European patent 0151470 the purification of the broth after separating of the impurities having a molecular weight above 50 000 dalton, is subjected to ultrafiltration or reverse osmosis using a membrane providing a separation cut at about 1000 dalton. The membranes employed are said to be stable at pH 1.5 - 2.2. The organic impurities having polarities lower than that of citric acid and molecular weights between 200 and 1000 dalton are then removed using a non-ionic adsorber resin with a specific surface of 300 to 1000 m^2/g. A disadvantage of this process is that membranes having a separation cut as low as 1000 dalton offer a high

resistance to the flow of the permeate so that for attaining reasonable throughput rates it is necessary to employ excessively large membrane areas and energy inputs for pumping the permeate through the membranes. Such membranes also clog after relatively small volumes of throughput.

The same disadvantages apply to Japanese Patent KOKOKU No 92071569, according to which ultrafiltration is performed to separate e.g. citric acid from sugars, all having a molecular weight below 1000 dalton.

German patent application 2 450 670 discloses a similar process for separating citrates and citric acid and/or isocitric acid from organic and inorganic contaminants using a membrane selective towards citric acid and citrates.

The same disadvantages apply also to nanofiltration. Nanofiltration denotes a form of ultrafiltration, wherein the substances being separated are of very low molecular weight. PCT/US 95/12160, filed and published after the earliest priority date of the present application relates to a process for the purification of citric acid which includes separating citric acid from residual sugars of the broth by nanofiltration. According to the disclosure the broth leaving the fermenter can contain 0.2% (w/w) unfermented fructose. The separation of citric acid from sugars by nanofiltration, is relatively incomplete. A relatively large proportion of the citric acid remains in the retentate and a substantial proportion of the sugars remains in the permeate, so that (besides ion exchange and activated carbon absorption) a mere repeated crystallisation of the permeate may not suffice to produce highest quality crystalline citric acid.

It is apparent from the aforesaid prior art that one of the main problems in the manufacture of citric acid of high purity is the presence of unfermented residual sugars in the fermentation broth and the separation thereof in the purification.

None of the aforegoing prior art specifically addresses the further problem of contamination with noxious organic acids, in particular toxic oxalic acid.

Attempts are continually made to improve the conversion ratio and thereby the yield in the fermentation of sugars to citric acid, which in turn leads to reduced residual concentrations of sugars in the broth. The problem is linked to the fact that A. niger as a rule ferments most effectively at neutral pH and sooner or later stops fermenting as the citric acid concentration increased and pH is lowered. GB patent 1 499 093 discloses a method of citric acid production with a strain of A. niger specifically developed by mutation and selection for improved citric acid yields. The strain is also described to be antagonistic to and inhibit the growth inter alia of acid-forming bacteria. A 99% w/w conversion to citric acid is claimed in neutral media (pH 6.8 - 7.2). However, the residual sugar content is still 0.5% w/w and the content of the broth of contaminating acids, including oxalic acid is 2 - 3%. This is due to the fact that neutral pH is also the ideal pH for the formation of contaminating noxious acids.

Techniques for producing new strains of A. niger for citric acid fermentation by mutagenesis, protoplast fusion and hybridisation are described by L. Martinková et al. "Protoplast fusion in Aspergillus niger strains accumulating citric acid", Folia Microbiol (Prague), 1990 35(2), 143-8.

There exists a need for a process for the manufacture of citric acid of high purity which overcomes or mitigates the abovementioned disadvantages. On the one hand there exists a need for a process which at least partly eliminates the use of chemicals in accordance with the prior art, eliminates or reduces the need for disposing of gypsum, is capable of producing a high conversion of carbohydrates into citric acid and a high yield of citric acid, wherein losses of starting material and desired products are minimised and which is capable of being performed economically advantageously.

A need further exists for a process wherein a fermentation broth is produced of such quality that by physical separation of high molecular weight contaminants, augmented by optionally conventional ion exchange and adsorption followed merely by repeated crystallisation of free citric acid, a high quality crystalline citric acid, more particularly of pharmaceutical grade can be obtained. To achieve this, the fermentation broth must inter alia have a low content of or be substantially free of noxious organic acids, in particular

toxic oxalic acid.

For reasons which will become clear from what follows, there exists a need for a strain of A. niger capable of fermenting carbohydrate substrates to lower sugar levels and higher citric acid concentrations. The resulting fermentation liquors should have low residual contents of readily carbonisable substances (RCS) and have low or be substantially free of noxious organic acid, in particular oxalic acid contamination.

There also exists a need for a process capable of achieving these objectives and which at least partly eliminates the use of chemicals in accordance with the prior art, eliminates or reduces the need for disposing of gypsum, is capable of producing a high conversion of carbohydrates into citric acid and a high yield of citric acid, wherein losses of starting material and desired products are minimised and which is capable of being performed economically advantageously.

In particular, there exists a need for a method and means for the manufacture of crystalline citric acid in an economic and ecologically advantageous manner, such citric acid being of a grade as required especially in the food industry, pharmaceutical industry, beverage industry and other industries.

Finally, there exists a need for a process and apparatus permitting the preparation of a raw citric acid fermentation product and purifying this by physical separation means alone.

DISCLOSURE OF THE INVENTION

In accordance with the present invention there is provided a process as set out in the opening paragraph wherein

a) a raw aqueous citric acid solution is produced wherein contaminants include not more than 2 000 mg/l of sugars and a ratio of citric acid to sugars exceeding 100 : 2;

b) the said preliminary physical separation of the citric acid from the bulk of its mainly macromolecular contaminants is performed using a separation device having a separation cut in the range between 5000 and 20 000 dalton.

The invention is based on the concept of initially preparing a fermentation broth having a low residual sugar content, a high ratio of citric acid to residual sugar and generally a low content of low molecular weight organic contaminants, so that by physical separation according to molecular weight employing a separating cut at a relatively high molecular weight of 5000 - 20 000 dalton a solution is obtained from which a high purity citric acid can be obtained by straight forward crystallisation of the free acid.

More particularly step b) is preferably performed by ultrafiltration.

Surprisingly the applicants found it possible to achieve an adequate preliminary purification by employing a separation cut (also known as separation line) in the range of 5000 to 20 000, preferably 10 000 dalton. This is considerably higher than the separation cut of 1000 dalton or even lower employed in the prior art. This results in greatly improved throughput rates and energy efficiency.

By adequate control of the quality of the fermentation broth, it was possible to limit impurities of molecular weight below the separation cut sufficiently (about 0.1 - 0.15 g/l) to make this possible.

Surprisingly it is possible, provided the sugar content is low as defined above, to separate by purely physical means more particularly by ultrafiltration a citric acid solution from which desired high purity citric acid or citric acid concentrate can be recovered by relatively simple and straightforward purification steps.

More particularly the step of prior separation, more particularly direct physical separation more particularly ultrafiltration is performed with the citric acid in a non-neutralised form. Accordingly, preferably step b) is performed at a pH of below about 2, in particular 1.6 or less, e.g. 1.4 - 1.5, using an ultrafiltration device, i.e. a separation membrane, sufficiently

stable at such pH and, by virtue of its aforesaid separation cut, capable of retaining macromolecular substances having a molecular diameter greater than a value between 5000 and 20 000, preferably greater than 10 000, whilst allowing substances of lower molecular weight, e.g. any residual non-fermented sugars to permeate. This means that the ratio of citric acid to sugars in the permeate must be as high as possible. Preferably the ultrafiltration device has the said separation cut when used at a temperature of 20 to 45°C and at a pressure not exceeding 2 MPa. More particularly the process is so performed that a filtrate of the ultrafiltration is obtained with a citric acid content exceeding 100 g/l, more particularly exceeding 130 g/l, preferably up to about 170 g/l, a sugar content of not more than 2000 mg/l and preferably considerably lower and a residual content of readily carbonisable substances (RCS) not exceeding 15 U, preferably not exceeding 13 U, and that this solution is subjected to a step of direct citric acid crystallisation.

These readily carbonisable substances (RCS) are generally formed from the reducing sugars at elevated temperatures, e.g. above 60°C as occurs in process steps such as are performed e.g. in evaporators. They are determinable by the Maillard reaction.

To obtain BP quality citric acid monohydrate crystals, RCS has to be in the range of 9-15 U. RCS values in the range of 9-13 U have been readily attained by the present invention.

Preferably even prior to the preliminary physical separation e.g. ultrafiltration, the RCS content should not exceed 170 U.

In this manner the subjection of the preclarified fermentation liquor to conventional precipitation of citric acid with lime in the form of calcium citrate is avoided.

An important preferred feature of the invention therefore resides in that the raw aqueous citric acid solution is produced by the fermentation of a carbohydrate substrate until its sugar level is reduced to not more than 1000 mg/l and its citric acid content exceeds 130 g/l, preferably 150 g/l, being for example as high as about 165 g/l. For the economics and effectiveness of the process it is thus very important that the fermentation

itself is performed so effectively that the sugar level is reduced to not more than 1000 mg/l, preferably to less than 800 mg/l, e.g. 600 mg/l (and even as low as 500 mg/l), in spite of the aforesaid relatively high citric acid content, being achieved and the resultant comparatively high acidity of the fermentation liquor. This amounts to a ratio of citric acid to sugars in the broth in excess of 130 : 1, preferably in excess of 165: 1, more particularly in excess of 187 : 1 and even as high as 275 : 1 to 300 : 1. This can be achieved by performing the fermentation using suitable selected Aspergillus niger in a manner as more fully set out below and by using a substrate of controlled quality.

It is furthermore an important preferred feature that the fermentation is commenced at a pH not exceeding pH 4 preferably not exceeding pH 3 and more preferably at a pH of 2.8 or less. By commencing the fermentation at such relatively low pH, undesirable parasitic fermentation reactions resulting in undesirable byproducts or contaminants such as oxalic acid and/or other undesirable acids are suppressed from the outset resulting not only in an improved conversion of starting material into desired products but also reducing subsequent purification problems, i.a. by a substantial or even total absence of oxalic acid.

To achieve the aforegoing the invention teaches the combined employment of particularly suitable strains of Aspergillus niger in combination with a substrate well adapted to such strains.

The substrate is a starch hydrolysate, e.g. starch hydrolysate produced with bacterial or fungal amylase or glucoamylase at 45 - 50°C or sucrose solution, preferably a sucrose solution which may optionally be of brown sugar quality, and the Aspergillus niger conidia used is a strain having essentially the aforesaid performance characteristics.

Obviously it is economically advantageous to be able to employ commercial grades of sugar which are only partly refined. Indeed, the strains of micro organism preferred for use in accordance with the invention derive nutritional benefits from the presence in the substrate of the "impurities" present in grades of sugar which have not been fully refined.

Likewise it is of considerable economic advantage that the process can be performed with a hydrolysed starch substrate, wherein the starch may be derived from a large variety of commercial starch sources. For example the hydrolysed starch substrate may be derived from any suitable commercial source of starch such as maize (corn), wheat, rice or other cereals, potatoes, sago, yam, or cassava. For that purpose a preferred strain of Aspergillus niger is a strain known as CCM 8210, or a strain having essentially the same performance characteristics. CCM 8210 was deposited at Czech Culture Collection of Micro-organisms under that number on 15 November, 1995. Preferably, and particularly, if a starch based substrate is used, the strain is said No CCM 8210 as such. Inventiveness is claimed for that strain as such, and this will be dealt with more fully below. In particular that strain has the property of being able to start the fermentation at a surprisingly low pH, thereby substantially suppressing the formation of oxalic acid and other noxious acids. This strain also offers the advantage that it can be used for fermenting starch-containing sugar juices e.g. derived from sugar cane having a substantial starch content.

However, the invention is not restricted to that strain but covers the use of other strains if capable of reducing the particular substrate to the required parameters. More particularly, if the fermentation is performed on a sugar substrate, more particularly a refined or partly refined sucrose solution, a commercial strain known as B-64-5 may be used.

To obtain BP quality citric acid monohydrate crystals, RCS has to be in the range of 9 - 15 U. RCS values in the range of 9-13 U have been readily attained by the present invention.

A further preferred aspect of the invention provides that after said step of direct crystallisation an amount of mother liquor from the direct crystallisation equivalent to from about 5 to 15% of the total citric acid formed by the fermentation is withdrawn and passed to separate processing. Preferably the amount of mother liquor withdrawn is equivalent to 7 to 13% of the total citric acid, more preferably about 10% of the total citric acid. Advantageously the mother liquor withdrawn is subjected to steps for the recovery of citric acid:

a) in purified form by calcium citrate precipitation, or

b) as sodium citrate, or

c) as liquid technical grade citric acid,

or more than one of these.

Even if modification a) is applied the amount of citric acid subjected to conventional precipitation with lime is but a small fraction of the total. The gypsum disposal problems are likewise reduced.

Normally, such balance of the mother liquor which is not withdrawn and passed to separate processing is recycled to the said direct crystallisation step. In the preferred process prior to a step of direct crystallisation substantially as above the solution obtained from the preliminary separation is subjected to purification by absorption and/or ion-exchange, preferably both. Preferably the crystallisation product of said direct crystallisation steps is subjected to at least one further purification step involving direct recrystallisation of citric acid in its non-neutralised form.

Preferably, prior to a step of direct crystallisation of citric acid substantially as described above the solution obtained from the preliminary separation is subjected to purification by absorption and/or ion-exchange. Also where as described above a portion of the mother liquor from this step of direct crystallisation is withdrawn and passed to separate processing, the balance of the mother liquor is recycled to the said crystallisation.

Preferably the crystallisation product of said crystallisation step is subjected to at least one further purification step involving direct recrystallisation.

The invention also provides an apparatus for carrying out the process, including the features as set out in the opening paragraph, comprising, in accordance with the invention the novel feature that the downstream preliminary separation stage is a physical separation device and wherein more particularly the physical separation device is an ultrafiltration device, having a separation cut in the range 5 000 - 20 000 dalton, more particularly

10 000 dalton, more particularly when used at a temperature of 20 to 45°C and at a pressure not exceeding 2 MPa.

The apparatus more particularly comprises a direct crystallisation stage for crystallising citric acid and separating it from its mother liquor, followed downstream by at least one further purification stage and optionally a passage for withdrawing part of the said mother liquor and feeding said part to separate and different citric acid recovery means and a passage for recycling remaining mother liquor to the said direct crystallisation stage.

A membrane device having those characteristics and stable at pH 1.2 has been developed according to the applicant's specification and is manufactured by Unipektin A.G., Zürich, Switzerland. However, any other suitable ultrafiltration device satisfying the aforesaid requirements regarding stability in acid medium and ability to retain macromolecular impurities, may be employed. From the preferred ultrafiltration step, using an ultrafiltration medium as aforesaid, a permeate may be obtained containing not more than 0.03 g/l of proteins.

Preferably said at least one further purification stage includes a further direct crystallisation stage, wherein citric acid is recrystallised.

The aforesaid separate and different citric acid recovery means (for the withdrawn part of the said mother liquor) includes a calcium citrate precipitation stage or a sodium citrate recovery stage or a liquid technical citrate recovery stage or a plurality of these.

Also in accordance with the present invention there is provided a new strain of Aspergillus niger, capable of fermenting carbohydrate substrates to citric acid, more particularly suitable for the above-described process, characterised by

a) its ability to continue the fermentation until the residual sugar level of the substrate has dropped to not more than 1000 mg/l,

b) its ability to start said fermenting at a pH at least as low as 4, preferably at least as low as 3,

c) its ability to continue fermentation until the pH of the substrate has dropped to at least as low as pH 1.4.

More particularly the new strain has the ability to continue the fermentation until the residual sugar level of the substrate has dropped to less than 800 mg/l, e.g. 600 mg/l and to start said fermenting at a pH at least as low as pH 2.9, preferably at a pH at least as low as pH 2.8.

Preferably the new strain has the ability to continue fermentation until the pH of the substrate has dropped to at least as low as pH 1.3, preferably 1.2.

The preferred embodiment of the strain has the ability to ferment a starch or sugar substrate, optionally of brown sugar quality, to a citric acid solution capable of being further purified for citric acid recovery by ultrafiltration without prior calcium citrate precipitation.

The preferred embodiment of the strain is capable of producing a fermentation broth wherein the ratio of citric acid to residual sugar exceeds 187.5 : 1 and may be as high as 275 : 1 to about 300 : 1.

The preferred embodiments have the ability to ferment saccharose, glucose, maltose, starch hydrolysates, lignified starches and cereal flours such as wheat flower to citric acid.

As aforesaid, the preferred strain substantially has the characteristics of Aspergillus niger strain No CCM 8210. That latter strain No CCM 8210 has been deposited at Czech Culture Collection of Micro-organisms, Česká sbirka mikroorganismù Masarykovy univerzity, Tvrdého 14, 602 00 BRNO, under accession No CCM 8210 on 15 November, 1995. More preferably, the strain used is, in fact, the said strain No CCM 8210 as such.

The strain was developed from an earlier strain obtained using protoplasts isolation from selected A. niger. The protoplasts were isolated from the hyphae with helical gastric juice in stabilised aqueous solution (0.7 MNaCl+glucose) and addition of calcium ions. The protoplasts were separated by filtration and washed with water. Suspensions of the protoplasts were radiated with UV (5.9 $J/m^2/sec$) for 5 minutes. Protoplast fusion was performed with wild strains selected for high growth and production ability, performed in a polyethylene glycol-calciumchloride system.

This A. niger strain was subjected to further mutation by a combination of UV radiation and chemical mutagens, i.e. 5-bromouracide, 2-aminopurine, diethylsulphate, ethylethane sulphonates and combinations of these. From this strain CCM 8210 was arrived at by selection of the high producing mutants, cultivated on sugar media. Bromcresol green was used as an indicator of acids formation. Selected monospore cultures were cultivated on media having high citric acid concentration in order to select strains tolerant to citric acid and low pH.

The invention includes the use of a strain as aforesaid for the production from a carbohydrate fermentation broth of a citric acid fermentation liquor purifiable by purely physical means, in particular ultra filtration to a solution from which substantially pure citric acid can be recovered by direct crystallisation.

The invention also provides a process and an apparatus as set out in the opening paragraph, wherein the fermentation is carried out with a strain as described above.

Due to the characteristics of the strain it has become practical (and this is indeed preferred) that the preliminary separation is performed by direct physical separation of the dissolved citric acid from contaminants of larger molecular size or weight.

Due to the characteristics of permeability and selectivity of proteins and macro-molecular substances from the citric acid liquor takes place, resulting in an aqueous citric acid solution containing in practice 10 to 20% by weight citric acid, 0.1-1.0% by weight proteins and percentages of reducing sugars as low as described above. The strain is ideally

suitable for and employed in a process as more fully described above.

BRIEF DESCRIPTION OF THE DRAWINGS

In the following the invention will be further described by way of example, partly with reference to the accompanying drawing which represents a block flow diagram of the process and of the apparatus for carrying out the process.

DETAILED DESCRIPTION OF SPECIFIC EMBODIMENTS

The strain Aspergillus niger CCM 8210

The above strain to be used in the preferred embodiment was deposited at the Czech Culture Collection of Micro-organisms on 15 November, 1995. One of its main characteristics is the ability to metabolise mono- and disaccharides and their mixtures and in particular also those resulting from the hydrolysis of pure starch and of starch-based materials by high conversion into citric acid by submerged culture.

Whereas prior art strains produced parallel to the citric acid small but still objectionable amounts of other organic acid, the strain CCM 8210 is capable of commencing fermentation at low pH, namely as low as pH 2.8 or less, preferably pH 2.5 - 2.7 or less where the formation of such undesirable organic acids, notably oxalic acid, is suppressed.

The strain has a higher conversion speed than conventional strains, namely of 1.3 g citric acid per 1 litre of fermentation broth per 1 hour.

The strain produces 90-93 kg of citric acid from 100 kg of sugar.

The new strain has high amylolytic activity in the range of pH 2.0 - 2.5, enabling the strain to ferment starch-based materials having a low dextrose equivalent (DE= 7 - 10%).

The strain is grown on potato glucose agar slant. After 7 days of cultivation, colonies are

formed up to 6.0 cm in diameter with total sporulation. The bottom side of the culture is cream yellow. The spores are brown-black and have a size of 5 - 6 μm. The viability of the spores by cultivation on the abovementioned agar slant is 3 months in terms of preserved production properties. In lyophilised form the viability is preserved for 3 to 5 years.

Referring now to the drawing, the following is a concise description of the preferred embodiment of the apparatus in accordance with the invention. For still further details reference should be made to the subsequent description of preferred embodiments of the process when carried out with the apparatus.

In the drawing the block denoted as 0 is normally not part of the apparatus proper, that is to say, it is usually operated separately in a microbiological laboratory facility where spores of a selected A niger strain are activated under sterile conditions in advance of being fed into the seed fermenter 1. The seed fermenter 1 includes an agitator operated with sterile air. It is followed downstream by a main fermenter 2, likewise equipped with agitating and sterile aeration means and operating under elevated pressure. Whereas the plant as a whole is designed in the preferred embodiment to operate substantially continuously, the seed fermenter 1 and the main fermenter 2 are preferably designed to operate batchwise, and for that reason a commercial plant will normally have a plurality of fermenters 1 and 2 in parallel, each fermenter being operated in a stage of the fermentation process different from the others so that when one fermenter approaches the end of the fermentation in that section of the process, another one is at a less advanced stage, thus permitting the remainder of the process to be operated continuously or semicontinuously. The fermenters 1 and 2 are supplied with sterilised water through sterilising filtration device 3 and with sterile nutrients solutions through a sterilising filtration device 4. In order to control foaming they are supplied with anti-foaming agent from an anti-foam agent source 5 and with substrate e.g. sucrose solution through a continuous sterilisation device 6 having a steam supply 8, a cooling water inlet 7 and a cooling water outlet 9.

The main fermenter(s) 2 is/are followed downstream by a sweep sieve 10 and a multi-leaf pressure filtration plant 11 cooled with cooling water 13 and from where mycelium is

collected at 12. The mycelium separation 12 preferably includes a belt filter on which the mycelium is freed of residual citric acid by rinsing and pressing in countercurrent, the citric acid rinsing liquor being recycled to the sieve and/or filtration stages 10, 11. An optional filtration bypass line 14 is shown leading from upstream of the pressure filtration stage 11 to downstream thereof.

The main pressure filtration stage 11 is followed downstream by a prefilter stage 15 for fine filtration and clarification prior to the stage constituting one of the main features of one aspect of the present invention and serving for the preliminary separation of citric acid from major, mainly macromolecular contaminants, being adapted for such separation to be performed by direct physical separation of the dissolved citric acid from contaminants of larger molecular size or weight. In the preferred embodiment, this stage denoted as 16 takes the form of an ultrafiltration device capable of retaining macromolecular substances, in particular proteinaceous byproducts of the fermentation, having a molecular weight greater than 10 000 Dalton. In order to be able to perform this task on the clarified fermentation broth containing the citric acid substantially in a non-neutralised condition, the ultrafiltration device must be stable in such acidic medium, more particularly at a pH of below 2 and preferably at a pH at least as low as 1.2. A suitable ultrafiltration membrane, satisfying these conditions has been developed in accordance with specifications designed by the present applicant and is available from Unipectin A.G. in Zürich. The use of such ultrafiltration membrane in a process as set out herein is considered to be within the scope of the present invention.

In practice a battery of ultrafiltration units in series is provided each comprising a circulation pump, an ultrafilter and a cooler, each successive module being connected to receive the overflow from the preceding module. The modules are connected to a supply of tap water 18 for backwashing from time to time. The filter cake consisting mainly of protein concentrate is collected at 17.

A duct 19 for collecting the permeate from the ultrafiltration device feeds into a set of carbon columns 21 charged with activated charcoal for the decolourisation of the citric acid solution and the absorption of any absorbable impurities. In practice three carbon columns

at a time are connected in series whilst a fourth column is subjected to regeneration or standby. Downstream of the carbon columns a battery of cation exchange columns 22 is provided connected to a source of demineralised water 23 and a source of regeneration solution 24 and an eluate discharge line 25. In practice two cation exchanger columns are connected in series at any time and a third column is not connected whilst being regenerated or on standby.

The cation exchanger battery 22 is followed downstream by an anion-exchanger column battery 26 which again is connected to a source of demineralised water 27 and a source of regenerating solution 28 and a discharge line 29 for eluate. Again two columns are connected in series at any one time whilst a third column not connected is subjected to regeneration or is on standby.

The ion-exchanger columns feed into a first direct crystallisation stage comprising an evaporator 30 heated with steam 31 and from where vapour 32 is discharged. The evaporator is followed downstream by a first crystalliser 40 and a first centrifuge 42. The first crystalliser 40 includes a steam feed duct 41 for operating a steam ejector (not shown) which generates a vacuum in the crystalliser 40. The first centrifuge 42 includes a supply 43 for demineralised water and an outlet for rinsing water 47 as well as an outlet 44 for mother liquor from the centrifugation. This outlet 44 is divided into a branch 46 which returns mother liquor to the first crystalliser and another branch 45 which bleeds off a portion of the mother liquor to a separate purification stage which will be described more fully further below.

The first centrifuge 42 feeds the crystals from the first direct crystallisation stage into a diluting vessel 48 supplied with demineralised water 49 and followed downstream by a second crystallisation stage comprising a second crystalliser 50 again supplied with steam 51 for a steam ejector (not shown) and including a vapour discharge 52 and a second centrifuge 53 with a supply of demineralised water 54 a mother liquor outlet 55 and a rinsing water outlet 58. The mother liquor outlet 55 branches into a duct 56 which returns part of the mother liquor to the second crystalliser 50 and a further duct 57 which returns the balance of the mother liquor to the first crystalliser 40. The solids collected in the

second centrifuge pass into a drier 59 supplied with drying air 60 and a recycling duct 61 for recycling oversize (agglomerated) particles back to the diluting vessel 48.

The drying section 59 is followed downstream by a sorting section 62 where classification takes place by sieving into desired fractions. These fractions are passed to the packing section 63.

Returning now again to the abovementioned further purification section supplied by the mother liquor duct 45 from the first crystallising section, this is basically a calcium citrate precipitation purification section and may be designed in a substantially conventional manner except for the optional omission of protein precipitation means. The section comprises a precipitation section 70 with feed means for lime milk 71, $Ca(OH)_2$. The precipitation stage feeds into a filtration stage 72 which includes a withdrawal line for filtrate (mud) 73. The filtration stage 72 includes a belt filter divided into four sections of which the last one is used for cleaning the belt. The remaining sections serve for separating the precipitate and for washing. The precipitated calcium citrate is fed into a decomposition section 74 having a supply of sulphuric acid 75.

Downstream of the decomposition section 74 there follows a filtration section 76, once again including a belt filter having four sections as aforesaid for separating the solution of citric acid from precipitated gypsum 78. The filtrate from section 76 passes through a final filtration section 77 where further gypsum 79 is withdrawn. The clarified filtrate 80 may be converted into sodium citrate in a manner known per se 81 or it may be concentrated and marketed as a liquid concentrate of citric acid 82 or it may be returned to duct 20 and recycled onto the activated carbon columns 21.

The above described apparatus is used as follows to perform the process.

Spore activation

This part of the fermentation process is performed on a laboratory scale in the microbiological laboratory in a sterile activating medium prepared in the usual manner from food grade sucrose (150 g/l), and citric acid monohydrate (2.0 g/l) in tap water. The pH is 2.5 and a temperature of 34±1°C is maintained. The A. niger of the selected strain, e.g. CCM 8210, are activated for about six hours in a biological incubator until the desired degree of swelling of the spores is observed microscopically.

Preparation of solutions for seed fermenter 1 and main fermenter 2

Carbohydrate solutions and nutrient solutions are prepared having the compositions described in the working examples.

The carbohydrate solution is first heated up to 100°C, then further sterilised at 140°C and cooled to 50°C before being fed into the respective fermenters 1 and 2.

The nutrient solutions are brought to the desired concentrations by dilution with drinking water and sterilised by sterilising filtration (3, 4).

Vegetative inoculum preparation (1)

The sterile hydrocarbon solution and, nutrients solutions and any required sterile filtered drinking water are dosed into the seed fermenters. The pH is adjusted to pH 2.5 with concentrated sulphuric acid. The sterile broth is inoculated with the activated spore suspension. When necessary and after a required time as determined by microscopical observation of morphological changes of spores small amounts of sterile potassium ferrocyanide solution are added to stimulate such biosynthetic activity of the culture which leads to an optimum citric acid production rate. Cultivation of the vegetative inoculum proceeds for about 48 hours with optimum agitation and aeration with sterile filtered air, sterile antifoaming agent being added when needed for foam control.

Main fermentation (2)

The exact required volumes of sterile carbohydrate solutions are placed in the main fermenters 2, the final carbohydrate concentration being adjusted by addition of sterile

filtered drinking water. Nutrients are dosed in and the pH is adjusted to pH 2.5 with concentrated sulphuric acid, to the same level as in the seed fermenters, in order to suppress undesired side reactions such as oxalic acid formation.

The broth is fully saturated with oxygen by aeration with sterile air for 30 minutes. The temperature is adjusted to 32°C and vegetative inoculum is admitted from the seed fermenters using elevated air pressure. The main fermentation proceeds with controlled agitation and aeration under a slightly elevated pressure of 0.3-0.5 bar, pH to drop naturally, a pO_2 of not less than $1mgO_2/l$ and a temperature of 32°C.

The substrate composition, biomass and product concentration and carbon source conversion are constantly monitored. Microscopic observations are also made. Over the fermentation period of about 144 hours the substrate concentration is continuously decreased, until at the end a sugar concentration of less than 0.8 g/l and a citric acid concentration of at least 130 g/l is reached. In practice the sugar content may drop to as low as 0.6 g/l and the citric acid (monohydrate) may reach 165 g/l.

Filtration, ultrafiltration (10, 11, 15, 16)

After completion of the fermentation the content of the respective fermenter is discharged into citric acid collecting drums via the sweep sieve 10 by means of the excess pressure in the fermenter 2. The citric acid is then passed to pressure filtration through one of two leaf filter presses, while the second such press is cleaned of mycelium and any other solid debris and regenerated with water.

The filtrate is fed through the prefilter 14 into the cooled ultrafiltration plant 16. The pressure drop through the ultrafiltration membrane is monitored to determine the time for cleaning the membrane of protein while switching to another ultrafiltration unit.

The protein residue is freed of citric acid by rinsing with water in countercurrent on a belt filter.

Purifying citric acid solution (21, 22, 26)

The ultrafiltrate is decolorised on the battery of carbon columns 21 of which at any one time one column is regenerated with caustic soda (NaOH), reactivated with dilute sulphuric acid and washed with demineralised water.

The citric acid solution having passed through the carbon columns is decationised on two cation exchanger columns 21 in series while a third column is being regenerated and held on standby. The potassium content of the eluate of the first column serves to indicate cation saturation of the column. Regeneration proceeds with dilute sulphuric acid, a portion of which is recycled as preregenerating liquor.

The decationised citric acid solution is then deanionised by passing through two anion exchanger columns in series, while a third column is being regenerated with caustic soda and then held on standby. The sulphate anions content of the eluate serves as indicator for the column saturation.

The regeneration eluates from the carbon and ion exchange columns are collected for the recovery of offgrade citric acid solutions.

The deionised dilute citric acid solution enters the evaporator 30 where the solution is concentrated by heating with steam.

Evaporation, crystallisation, centrifugation

Concentration of the citric acid takes place by multiple stage evaporation until citric acid crystallises and is passed through a crystal overflow drum and a crystal suspension vessel via a hydrocyclone to be separated in the first centrifuge 42.

The crystals collected in the first centrifuge 42 are redissolved in a dissolving vessel. Mother liquor from the centrifuge 42 is mostly recycled into the first crystalliser 40. A small portion (about 10%) is withdrawn (45) as offgrade citric acid solution or for separate processing.

The citric acid from 42 redissolved at 48 is once again concentrated with heat and vacuum and passed, again through a crystal overflow drum and a crystal suspension vessel via a hydrocyclone (not shown), this time into the second crystalliser vessel 50 and from there into the second centrifuge 53. Centrifuged crystals are fed via a wet feeder (not shown) into the dryer 59. Mother liquor from the centrifuge 53 is partly recycled (55) to the second crystalliser 50 and partly (57) to the first crystalliser 40.

Drying, distribution

The dryer 59 is a wet conveyor dryer where the crystals are dried by hot air and then passed into a dry crystal separator 62 equipped with a dust separator and a classifier. There the crystals are sorted by sieving into three fractions. Oversize particles, e.g. over 2.0 mm are returned to the first crystalliser 40 to be redissolved and passed once again through the two crystallisation stages.

Throughout the process vented vapours are subjected to scrubbing to recover citric acid entrained in those vapours.

Processing of offgrade citric acid

Offgrade citric acid solutions, in particular the small portion of mother liquor (45) withdrawn from the first centrifuge 42 may now be treated, e.g. substantially in a conventional manner, by precipitation of calcium citrate with lime milk (70, 71). The lime milk is continuously dosed into the precipitation reactor 70 and heated with steam to 85°C to form a suspension of calcium citrate which is dropped onto a band filter 72, divided into four sections of which the last one is used for cleaning the band. In the first section water is separated from the suspension. The filter cake is then washed with hot water in countercurrent through successive sections, the water being finally sucked off under vacuum.

The dried filter cake drops into a decomposition vessel 74 where it is decomposed with sulphuric acid to form citric acid and precipitated gypsum. The solid gypsum is filtered off on a second band filter 76 similar to band filter 72. The gypsum filter cake is washed in countercurrent with water. In the third section the filter cake is washed with hot water.

The washing liquors from the second and third sections are recycled for washing of the cake.

The filtrate is passed through a final filtration 77 before (80) being recycled (20) to the top of carbon columns 19.

Alternatively the filtrate may be concentrated and marketed as a commercial grade citric acid solution.

Conidia Production and Conservation

Preserving strain activity

The activity and morphological integrity of the selected strain is preserved in a socalled "spore conserve", which is a mixture of spores and carbon in a ratio of 1 : 1 - 2, stored at 12 - 20°C and a relative humidity of not more than 70%.

Conidia cultivation

Depending on the intensity of consumption, this is carried out in one or two stages

1st stage of cultivation:

The surface of agar in aluminium dishes is inoculated from the starting culture. The dishes are put into a cultivation car, first at 32°C for 8.5 days, when the relative humidity is decreased from 80 - 90% down to 60 - 70%. During that period the number of culture dishes increases from 4 - 6 initially to about 40 dishes. As from the 10th day the temperature of the car is kept at 20 - 25°C.

After 10 days morphological control is carried out on samples and non-acceptable dishes are discarded. From the remainder the spores are harvested and stored as aforesaid.

After microbiological and biochemical testing they are used in the 2nd stage of cultivation.

2nd stage of cultivation:

This is done under the same conditions as in the 1st stage.

Spores are taken out and dried on stainless steel dishes of 27 ± 1°C to result in a 20 - 25% weight loss.

The dried spores are mixed with agitation with active carbon in a ratio of 1 : 1. This mixture is used for the fermentation process.

Example 1

Corn starch hydrolysate with DE=15% is prepared and passed into a fermentation medium containing per litre:

sugar	180g
ammonium sulphate	2.8g
potassium dihydrogen phosphate	0.3g
calcium chloride	0.15g
zinc sulphate	0.08g

100 litre of the sterile cultivation medium, pH 2.7 - 2.8, is inoculated with 2 - 3 vol% of 40 - 48 hour inoculum of A. niger CCM 8210 and is cultivated at 32 - 33°C for 155 - 160 hours at a tangential impeller tip speed up to 5.2 $m.s^{-1}$ and an aeration rate of 0.2 - 0.35 VVM (volume of air per volume of bioreactor per minute).

The final concentration of impurities in the fermentation broth after biomass and prefiltration is in the range 2.0 - 3.0 g/l.

This broth is treated as above described with reference to the drawing. The concentration of impurities smaller than 10 000 dalton in the permeate of the ultrafiltration is in the range 0.1 - 0.15 g/l, being mostly pigments (aspergillin), peptides (products of protein hydrolysis) and saccharides (8 glucose units and higher).

The residual unfermented sugar content is 650 mg/l. Oxalic acid was completely absent.

The citric acid yield was 165g.l^{-1}.

Example 2

A fermentation liquor is prepared from hydrolysed maize meal with DE=40%, containing per litre:

sugar	180g
ammonium nitrate	2.0g
potassium dihydrogen phosphate	0.2g
calcium chloride	0.1g

The sterile medium is inoculated at pH 2.8 - 3.0 in a 15m^3 fermenter with 2 - 3 vol% of 40 - 48 hour A.niger CCM 8210 inoculum and is cultivated at 32% and an aeration rate of 0.25 - 0.35 VVM for 155 - 160 hours. Further treatment as in Example 1. Yield of citric acid 165g.l^{-1}. Oxalic acid was completely absent. Residual sugar 0,5 g/l.

Example 3

Composition of fermentation liquor, used analogously to Example 1 or 2:

liquefied corn starch, maltose or glucose syrup expressed as sugar 180 kg m^{-3}

corn starch *	180 kg.m^{-3}
ammonium sulphate	2.25 kg.m^{-3}
potassium dihydrogenphosphate	0.5 kg.m^{-3}
calcium chloride	0.15 kg.m^{-3}
zinc sulphate	0.05 kg.m^{-3}
copper sulphate	0.05 kg.m^{-3}

magnesium sulphate	0.5 $kg.m^{-3}$

*could be prepared as liquefied starch, maltose or glucose syrup in various concentrations.

Example 4

Composition of fermentation liquor, used analogously to Example 1 or 2:

sucrose	180 $kg.m^{-3}$
ammonium nitrate	2.5 $kg.m^{-3}$
potassium dihydrogenphosphate	0.25 $kg.m^{-3}$
zinc sulphate	0.02 $kg.m^{-3}$
copper sulphate	0.025 $kg.m^{-3}$
magnesium sulphate	0.25 $kg.m^{-3}$

Example 5

10 000 litres of citric acid-liquors after fermentation for citric acid, containing 150 g/l of citric acid, 1 g/l of proteins and 0.5 g/l of reducing sugars and no detectable oxalic acid are divided on ultrafiltration polymer membranes at temperature of 25°C and pressure of 1.6 Mpa into:

- 9 000 litres of permeate that contains 150 g/l of citric acid, 0.03 g/l proteins and 0.5 g/l of reducing sugars

- 1 000 litres of concentrate that contains 150 g/l of citric acid, 9.73 g/l of proteins and 0.5 g/l of reducing sugars.

The permeate (9 000 l) is subjected to decolourisation on active charcoal columns, deionisation on cation exchangers and anion exchangers and concentration in a vacuum evaporator to 850 g/l.

The concentrate is subjected to diafiltration resulting in:

- diafiltrate which is combined with the permeate from ultrafiltration

- diaconcentrate, from which pectinase is isolated as by-product.

By the method according to the invention, 1 425 kg of crystalline citric acid was obtained. The purity was determined by izotachophoresis with precision of 99 to 101%. Other parameters comply with the valid standard BP 93.

Example 6

A. niger strain B - 64 - 5

This strain (Commercially available: 1. Lesniak, W.: Dobor podloza fermentacyjnego dla nowo wyizolowanego wysokoaktywnego mutanta Aspergillus niger. Prace Naukowe AE Wroclaw, Technologia, 1975, 69, 93. 2. Lesniak, W.: Selekcja wysokoaktywnych szczepow Aspergillus niger dla fermentacji wglebnej kwasu cytynowego. Prace naukowe AE Wroclaw, Technologia, 1977, 188, 21.) is particularly suitable for the citric acid fermentation of refined sucrose.

The sucrose solution should have a concentration of approximately 50% w/w.

The nutrients are employed in 10% ww solutions to provide the following concentrations in the broth:-

potassium hydrogen phosphate	0.2 g/l
ammonium nitrate	2.0 g/l
magnesium sulphate	0.2 g/l

The citric acid liquors obtained by the above Examples 3, 4 and 6 are subjected to the purification process as described above.

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 14, line

B. **IDENTIFICATION OF DEPOSIT** Further deposits are identified on an additional sheet ☐

Name of depositary institution: Czech Collection of Mikroorganisms (CCM)
ul.Tvrdého c. 14
60200 Brno, Czech Republic

Address of depositary institution *(including postal code and country)*:

ul.Tvrdého c. 14
602 00 Brno /CZ/
Czech Republic

Date of deposit	Accession Number
15 November 1995 /15.11.1995/	CCM 8210

C. **ADDITIONAL INDICATIONS** *(leave blank if not applicable)* This information is continued on an additional sheet ☐

D. **DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE** *(if the indications are not for all designated States)*

E. **SEPARATE FURNISHING OF INDICATIONS** *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
☒ This sheet was received with the international application	☐ This sheet was received by the International Bureau on:
Authorized officer: Dr. Vladimír Banský	Authorized officer:

Form PCT/RO/134 (July 1992)

CLAIMS

1. A process for producing purified citric acid or citric acid concentrate which comprises producing by fermentation a raw aqueous citric acid solution and subjecting it to a preliminary physical separation of citric acid from the bulk of its major, mainly macromolecular contaminants and optional subsequent purification steps, characterised in that

   a) a raw aqueous citric acid solution is produced wherein contaminants include not more than 2 000 mg/l of sugars and with a ratio of citric acid to sugars exceeding 100 : 2; and

   b) the said preliminary physical separation of the citric acid from the bulk of its mainly macro molecular contaminants is performed, using a separation device having a separation cut in the range between 5000 and 20 000 dalton.

2. A process as claimed in claim 1, characterised in that step b) is performed by ultrafiltration.

3. A process as claimed in claim 1 or 2, characterised in that step b) is performed with the citric acid in a non-neutralised form.

4. A process as claimed in any one of claims 1 to 3, characterised in that the ultrafiltration device has a separation cut of about 10 000 dalton, when used at a temperature of 20 to 45°C and at a pressure not exceeding 2 MPa.

5. Process as claimed in any one of claims 1 to 4, characterised in that the physical separation is performed by ultrafiltration at a pH of from 1.4 to 1.5.

6. A process as claimed in any one of claims 1 to 5, characterised in that the filtrate of the ultrafiltration is obtained with a citric acid content exceeding 100 g/l, a sugar content of not more than 1 000 mg/l and a residual content of readily carbonisable

substances (RCS) not exceeding 15 U and that this solution is subjected to a step of direct crystallisation.

7. A process as claimed in any one or more of claims 1 to 6, characterised in that the raw aqueous citric acid solution is produced by the fermentation of a carbohydrate substrate until its sugar level is reduced to not more than 1000 mg/l, and its citric acid content exceeds 130 g/l, preferably exceeds 150 g/l.

8. A process as claimed in any one of the preceding claims, characterised in that the residual sugar level is reduced to less than 800 mg/l in the fermentation broth and the ratio of citric acid to residual sugar exceeds 187.5 : 1

9. A process as claimed in any one of claims 1 to 8, characterised in that the fermentation is performed, using a strain of Aspergillus niger and that the fermentation is commenced at a pH not exceeding pH 4.

10. A process as claimed in claim 9, characterised in that the fermentation is commenced at a pH not exceeding pH 2.8.

11. A process as claimed in any one or more of claims 1 to 10, characterised in that the fermentation is performed on a sugar substrate, more particularly a sucrose substrate, and the Aspergillus niger used is a strain known as B-64-5 or a strain having essentially the same performance characteristics.

12. A process as claimed in any one or more of claims 1 to 10, characterised in that the fermentation is performed on a hydrolysed starch or a sucrose solution as substrate and the Aspergillus niger strain used is a strain known as CCM 8210 or a strain having essentially the same performance characteristics.

13. A process as claimed in claim 12, characterised in that the substrate is a hydrolysed starch substrate and the Aspergillus niger strain used is a strain known as CCM 8210 or a strain having essentially the same performance characteristics.

14. A process as claimed in any one or more of claims 1 to 13, characterised in that subsequent to said preliminary separation of the citric acid from the bulk of its mainly macromolecular contaminants and after a further purification by direct crystallisation of citric acid an amount of mother liquor from the direct crystallisation equivalent to from 5 to 15% of the total citric acid in the raw aqueous citric acid solution is withdrawn and passed to separate processing.

15. A process as claimed in claim 14, characterised in that the amount of mother liquor withdrawn is equivalent to 7 to 13% of the total citric acid.

16. A process as claimed in claim 15, characterised in that the amount of mother liquor withdrawn is equivalent to about 10% of the total citric acid.

17. A process as claimed in any one or more of claims 14 to 16, characterised in that the mother liquor withdrawn is subjected to steps for the recovery of citric acid

a) in purified form by calcium citrate precipitation, or
b) as sodium citrate, or
c) as liquid technical grade citric acid,
or a plurality of a) to c).

18. A process as claimed in any one or more of claims 14 to 17, characterised in that prior to a step of direct crystallisation substantially as defined in claim 14 the solution obtained from the preliminary separation is subjected to purification by absorption and/or ion-exchange.

19. A process as claimed in any one or more of claims 14 to 18, characterised in that the balance of the mother liquor is recycled to the said crystallisation.

20. A process as claimed in any one or more of claims 14 to 18, characterised in that the crystallisation product of said crystallisation step is subjected to at least one further purification step involving recrystallisation.

21. An apparatus for carrying out a process as claimed in any one or more of claims 1 to 20 comprising a fermentation stage (1, 2) and optional separation means (10, 11, 15), capable of producing a raw citric acid solution, and a downstream preliminary physical separation stage, wherein citric acid is separated from the bulk of its major, mainly macromolecular contaminants, characterised in that the downstream preliminary physical separation stage (16) includes a physical separation device having a separation cut in the range between 5000 and 20 000 dalton.

22. An apparatus as claimed in claim 21, characterised in that the physical separation device (16) is an ultrafiltration device.

23. An apparatus as claimed in claim 22, characterised in that the ultrafiltration device (16) has the said retention properties when used at a temperature of 20 to 45°C and at a pressure not exceeding 2 MPa.

24. An apparatus as claimed in any one of claims 21 or 23, characterised in that the separation device (16) has a separation cut of about 10 000 Dalton.

25. An apparatus as claimed in any one of claims 21 to 24, characterised in that the physical separation device (16) is an ultrafiltration device, stable at a pH of 2 or less.

26. An apparatus as claimed in claim 25, characterised in that the ultrafiltration device (16) comprises a polymer membrane stable at a pH down to at least about 1.2.

27. An apparatus as claimed in any one of claims 21 to 26, characterised in that it comprises a direct crystallisation stage (30, 40, 42) for crystallising citric acid and separating it from its mother liquor, followed downstream by at least one further purification stage (48, 50, 53).

28. An apparatus as claimed in claim 27, characterised by a passage (44, 45) for withdrawing part of the said mother liquor and feeding said part to separate and different citric acid recovery means (70, 72, 74, 76, 77) and a further passage (80) for recycling remaining mother liquor to the said direct crystallisation stage.

29. An apparatus as claimed in claim 27 or 28, characterised in that said at least one further purification stage includes a further direct crystallisation stage, wherein citric acid is recrystallised.

30. An apparatus as claimed in claim 28, characterised in that said separate and different citric acid recovery means includes a calcium citrate precipitation stage (70) or a sodium citrate recovery stage or a liquid technical citrate recovery stage or a plurality of these.

31. The use of an ultrafiltration medium stable at a pH of 2 or less and having a separation cut between 5000 and 20 000 dalton for the separation of citric acid from its macromolecular contaminants in a citric acid fermentation broth.

32. A new strain of Aspergillus niger, capable of fermenting carbohydrate substrates to citric acid, more particularly for carrying out a process as claimed in any one of claims 1 to 20, characterised by

a) its ability to continue the fermentation until the residual sugar level of the substrate has dropped to not more than 1000 mg/l,

b) its ability to start said fermenting at a pH at least as low as 4,

c) its ability to continue fermentation until the pH of the substrate has dropped to at least as low as pH 1.4.

33. A strain as claimed in claim 32, characterised by its ability to continue the fermentation until the residual sugar level of the substrate has dropped to less than 800 mg/l.

34. A strain as claimed in claim 32 or 33, characterised by its ability to start said fermenting at a pH at least as low as pH 2.9.

35. A strain as claimed in any one or more of claims 32 to 34, characterised by its ability to start said fermenting at a pH at least as low as pH 2.8.

36. A strain as claimed in any one or more of claims 32 to 35, characterised by its ability to continue fermentation until the pH of the substrate has dropped to at least as low as pH 1.3.

37. A strain as claimed in any one or more of claims 32 to 36, characterised by its ability to ferment a starch or sugar substrate, optionally of brown sugar quality, to a citric acid solution capable of being further purified for citric acid recovery by ultrafiltration without prior calcium citrate precipitation.

38. A strain as claimed in any one or more of claims 32 to 37, characterised in that it substantially has the characteristics of Aspergillus niger strain No CCM 8210.

39. Aspergillus niger strain No CCM 8210.

40. A process for producing citric acid or citric acid concentrate by fermentation of a carbohydrate substrate with Aspergillus niger, followed by mycelium removal, a preliminary separation of citric acid from major, mainly macromolecular contaminants and subsequent purification steps, characterised in that the fermentation is carried out with a strain as claimed in one or more of claims 32 to 39.

41. A process as claimed in claim 40, characterised in that the preliminary separation is performed by direct physical separation of the dissolved citric acid from contaminants of larger molecular size or weight, using a separation device having a separation cut in the molecular weight rang e of 5000 to 20 000 dalton.

42. A process as claimed in claim 41, characterised in that the physical separation is performed with the citric acid in non-neutralised form.

43. A process as claimed in any one of claims 40 to 42 characterised in that the physical separation is carried out by ultrafiltration at a pH of not more than 1.6.

44. A process as claimed in claim 43, characterised in that the ultrafiltration is carried out at a pH of from 1.4 to 1.5.

45. A process as claimed in any one or more of claims 40 to 44, characterised in that it is carried out with an ultrafiltration membrane device stable at a pH of 2 and lower and having a separation cut at a molecular weight of about 10 000 dalton.

46. A process as claimed in any one or more of claims 40 to 45, characterised in that the fermentation subjected to said physical separation has a sugar content of not more than 1000 mg/l and a citric acid content of at least 130 g/l.

47. A process as claimed in claim 46, characterised in that said sugar concentration is less than 800 mg/l and the citric acid content is about 130 to 170 g/l.

48. A process as claimed in any one or more of claims 40 to 47, characterised in that from the physical separation step, a citric acid solution is obtained having a residual content of readily carbonisable substances (RCS) not exceeding 15 U and that this solution is subjected to a step of direct crystallisation of citric acid in its non-neutralised form.

49. A process as claimed in claim 48, characterised in that after said step of direct crystallisation an amount of mother liquor from the direct crystallisation equivalent to from about 5 to 15% of the total citric acid formed by the fermentation is withdrawn and passed to separate processing.

50. A process as claimed in claim 49, characterised in that the amount of mother liquor withdrawn is equivalent to 7 to 13% of the total citric acid.

51. A process as claimed in claim 50, characterised in that the amount of mother liquor withdrawn is equivalent to about 10% of the total citric acid.

52. A process as claimed in any one of claims 49 to 51, characterised in that the mother liquor withdrawn is subjected to steps for the recovery of citric acid

a) in purified form by calcium citrate precipitation, or
b) as sodium citrate, or
c) as liquid technical grade citric acid,
or more than one of these.

53. A process as claimed in any one or more of claims 49 to 52, characterised in that such balance of the mother liquor which is not withdrawn and passed to separate processing is recycled to the said direct crystallisation step.

54. A process as claimed in any one or more of claims 40 to 53, characterised in that prior to a step of direct crystallisation substantially as defined in claim 48 the solution obtained from the preliminary separation is subjected to purification by absorption and/or ion-exchange.

55. A process as claimed in any one or more of claims 40 to 54, characterised in that the crystallisation product of said direct crystallisation steps is subjected to at least one further purification step involving direct recrystallisation of citric acid in its non-neutralised form.

56. A process as claimed in any one or more of claims 40 to 55, characterised in that the citric acid fermentation liquor obtained by the citric acid fermentation is subjected to biomass removal followed by ultrafiltration on a polymer membrane having a separating line in the range of 5 000 to 20 000 Dalton at a temperature of 20 to 45% and a pressure not exceeding 2 MPa.

57. Process as claimed in any one of claims 1 to 20 or 40 to 56, characterised in that the fermentation step is performed under submerged fermentation conditions.

58. Apparatus for carrying out the process as claimed in any one or more of claims 1 to 20 or 40 to 57, characterised by an ultrafiltration installation (16) having a separation cut at a molecular weight in the range of 5000 to 20 000 dalton, followed downstream by a crystallisation stage for citric acid purification by direct crystallisation and a means for withdrawing a portion of the mother liquor from that crystallisation stage and feeding it into a separate citric acid recovery section.

59. The use of a strain as claimed in any one or more of claims 32 to 39 for the production from a carbohydrate fermentation broth of a citric acid fermentation liquor purifiable by ultrafiltration to a solution from which substantially pure citric acid can be recovered by direct crystallisation.

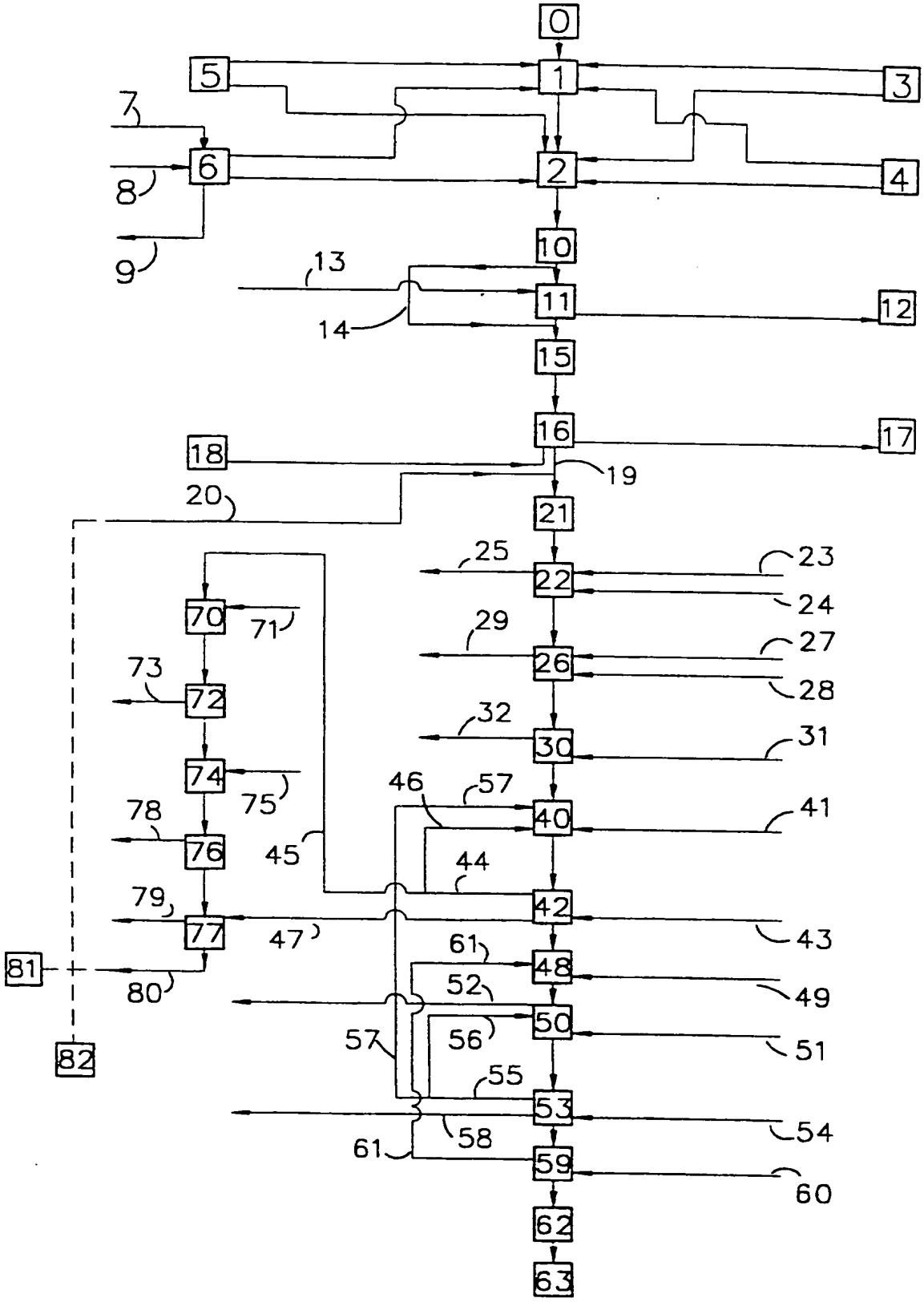
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FIG. 1

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

LikoSpol
Peter Michalík
Miletičova 23, P.O.BOX 4
820 06 Bratislava

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM

Identification reference given by the depositor:	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY
Aspergillus niger S - 1	**CCM 8210**

II. SCIENTIFIC DESCRIPTION AND/OR TAXONOMIC DESIGNATION

The microorganism identified under I. above was accompanied by:

( ) a scientific description
(X) a proposed taxonomic designation

(Mark with a cross where applicable)

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I. above, which was received by it on 15.11.1996 (date of original deposit)[1].

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I. above was received by this International Depositary Authority on (date of original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).

V. INERNATIONAL DEPOSITARY AUTHORITY

Name: CCM - CZECH COLLECTION OF MICROORGANISMS
Masaryk University

Address: Tvrdého 14
602 00 Brno
Czech Republic
tel. +42 5 337231
fax. +42 5 41211214

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):

Dr. Dana Hanuláková

Date: 12.02.1996

MASARYK UNIVERSITY BRNO Czech Collection of Microorganisms

[1] Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired.

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

LikoSpol
Miletičova 23, P.O.BOX 4
820 06 Bratislava

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: Peter Michalík Address: LikoSpol Miletičova 23 820 06 Bratislava	Name: **Aspergillus niger** Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: **CCM 8210** Date of the deposit or of the transfer[1]: **15.11.1995**

III. VIABILITY STATEMENT

The viability of the microorganism identified under II above was tested on[2] 23.11.1995
On that date, the said microorganism was

(X)[3] viable
( )[3] no longer viable

IV CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[4]

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: CCM - CZECH COLLECTION OF MICROORGANISMS Masaryk University Address: Tvrdého 14 602 00 Brno Czech Republic tel. +42 5 337231 fax. +42 5 41211214	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): Dr. Dana Hanuláková Date: 12.02.1996

MASARYK UNIVERSITY BRNO Czech Collection of Microorganisms

[1] Indicate the date of original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).
[2] In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.
[3] Mark with a cross the applicable box.
[4] Fill in the information has been requested and if the results of the test were negative.

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PC1/SK 96/00014

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12P7/48 C12M1/12 //C12R1:585

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12P C12M

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DE 35 02 924 A (BENCKISER GMBH JOH A) 8 August 1985	1-4, 17-19, 21,22, 25,27,31
Y	see page 5, paragraph 5 - page 6, paragraph 2; claims 1,11	1,5-7, 9-13,17, 32, 34-47, 52, 54-57,59
	see page 16, paragraph 2 - paragraph 3	
	see page 8, paragraph 1 - page 8, paragraph 2	
	see page 8, paragraph 5; example 3	

	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

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Date of the actual completion of the international search	Date of mailing of the international search report
5 February 1997	12.02.97
Name and mailing address of the ISA	**Authorized officer**
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Coucke, A

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PC1/SK 96/00014

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 3 335 067 A (M. BATTI) 8 August 1967 see claim 1; examples ---	1,5-7, 9-13,17, 32, 34-47, 52, 54-57,59
Y	US 2 394 031 A (S. WAKSMAN ET AL.) 5 February 1946 see column 2, line 51; claims 1,2,7,10 see column 3, line 19 - line 29 ---	1,5-7, 9-13,17, 32, 34-47, 52, 54-57,59
Y	US 3 886 041 A (KABIL ADEL J) 27 May 1975 see claims 1-3; examples -----	1,7, 9-13,17, 32, 34-47, 52, 54-57,59

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter nal Application No
PC1/SK 96/00014

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
DE-A-3502924	08-08-85	EP-A-	0151470	14-08-85
		JP-C-	1483101	27-02-89
		JP-A-	60199390	08-10-85
		JP-B-	63029999	16-06-88
		US-A-	4855494	08-08-89
US-A-3335067	08-08-67	NONE		
US-A-2394031	05-02-46	GB-A-	568508	
US-A-3886041	27-05-75	AT-A-	307346	15-04-73
		AU-B-	467172	20-11-75
		AU-A-	4561772	21-02-74
		BE-A-	787594	18-12-72
		CA-A-	978121	18-11-75
		CH-A-	566393	15-09-75
		DE-A-	2240052	22-02-73
		FR-A-	2149549	30-03-73
		GB-A-	1392942	07-05-75
		NL-A-	7211219	20-02-73